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Cell Identity Regulators Link Development and Stress Responses in the *Arabidopsis* Root

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SUMMARY

Stress responses in plants are tightly coordinated with developmental processes, but interaction of these pathways is poorly understood. We used genome-wide assays at high spatiotemporal resolution to understand the processes that link development and stress in the *Arabidopsis* root. Our meta-analysis finds little evidence for a universal stress response. However, common stress responses appear to exist with many showing cell type specificity. Common stress responses may be mediated by cell identity regulators because mutations in these genes resulted in altered responses to stress. Evidence for a direct role for cell identity regulators came from genome-wide binding profiling of the key regulator SCARECROW, which showed binding to regulatory regions of stress-responsive genes. Coexpression in response to stress was used to identify genes involved in specific developmental processes. These results reveal surprising linkages between stress and development at cellular resolution, and show the power of multiple genome-wide data sets to elucidate biological processes.

INTRODUCTION

Plant development is highly plastic and is profoundly influenced by the environment. Surprisingly, little research focuses on the interplay between development and environmental stress, and much of the work that has been done centers on the response of whole organs to a small number of stresses (Kreps et al., 2002; Rabbani et al., 2003; Kilian et al., 2007; Zeller et al., 2009). Previous work has identified several common transcriptional responses to stress (Fujita et al., 2006; Walley et al., 2007; Hirayama and Shinozaki, 2010), and has led to the idea that plants have a universal stress response (Walley and Dehesh, 2010; Ma and Bohnert, 2007). However, whole organs are a mixture of multiple cell types, and whole-organ transcriptional responses often obscure the more complex and subtle changes that occur at cell type resolution (Birnbaum et al., 2003; Brady et al., 2007; Gifford et al., 2008; Dinneny et al., 2008). Thus, how environmental stress affects the development pathways that regulate individual cell types is largely unknown. To address this issue requires examination of many different stresses at the resolution of individual cell types.

The *Arabidopsis* root is an excellent model for this purpose. Although 15 cell types have been described, the root can be simplified to a set of concentric cylinders that are radially symmetric around a central axis. From external to internal cell layers, these are the epidermis (Epi), cortex (Cor), endodermis (End), and stele (Stl), with the columella (Col) and lateral root cap providing additional layers at the root tip. The longitudinal axis of the root can be viewed as a developmental timeline, with young cells at the root tip in the meristematic zone and older cells distal to the tip in the elongation and differentiation zones.

Each cell type in the root has its own transcriptional profile (Brady et al., 2007), and a recent report demonstrated that cell identity plays an important role in a plant's response to environmental stress (Dinneny et al., 2008). However, this study was restricted to examining two stimuli, which limited the ability to identify patterns of gene expression within cell types across many stresses. To fully understand the root's response to stress requires multiple conditions at high resolution using the same cell types and developmental stages. Here, we profiled the transcript populations of whole roots, five cell types, and four developmental stages under two stress conditions: low pH and sulfur deficiency (−S). We combined these with two data sets of similar cell type resolution describing the root response to high NaCl and iron deficiency (−Fe) at high resolution (Dinneny et al., 2008), and with ten data sets that describe the whole-root response to ten different stresses (Kilian et al., 2007). This combined data set allowed us to search for common stress responses (CSRs) in whole roots and cell types, and to identify patterns of gene expression both within a given cell type across multiple stresses and across multiple cell types for a given stress.

Although we find CSRs in the whole root, we find little evidence for a universal stress response at either whole root or cell type resolution. We show that CSRs, such as those mediated by the plant hormone abscisic acid (ABA), exhibit cell type specificity, and we provide both mutational and genome-wide binding data that suggest that this specificity is mediated by cell identity regulators. We show that although different stresses uniquely

affect root spatiotemporal transcriptional programs, there is a set of genes enriched in specific cell types regardless of environmental stress, suggesting that cell-cell communication is an important part of the root's response to stress.

RESULTS

A CSR in Roots

We profiled the whole-root transcriptional response to pH 4.6 (low pH) and −S at different time points after transfer to the stress and combined these data with 12 publicly available time course (TC) data sets from whole roots (see Table S1 available online) exposed to different environmental stresses to try to identify a "universal stress response" in the *Arabidopsis* root. To determine whether a universal stress response exists in the root, we first identified differentially expressed genes in each of the 14 treatments using the RankProd package in R (Hong et al., 2006). The resulting p values were combined using Fisher's inverse χ^2 method and FDR values obtained. We required that significant genes have a combined FDR value of <0.0001 and have an FDR of <0.01 in at least 75% (11 of 14) of the conditions (see Experimental Procedures). Using these criteria, we identified eight genes that were activated and one repressed gene (Table S2). The activated genes include a DNAJ heat-shock protein and DREB2A, a transcription factor (TF) with major roles in drought stress and ABA responses (Sakuma et al., 2006). Consistent with the paucity of universally responsive genes, principal component analysis (PCA) of gene ontology (GO) categories significant under each stress showed that each stress elicited distinct functional responses in the root (Figure 1A).

Although the PCA did not suggest the presence of a universal stress response, clustering of some stresses indicated that there may be functions common to a smaller subset of stresses. Therefore, we examined the set of genes responsive in at least 7 of the 14 conditions, and called these the "common stress response" (CSR; Table S2). The 274 activated genes in the CSR are enriched for well-known abiotic and biotic stress responses, including "response to abscisic acid stimulus" ($p < 10^{-9}$) and "response to other organism" ($p < 10^{-5}$). Four transcription-related categories are also enriched within these genes, including "transcription factor activity" ($p < 10^{-6}$) and "DNA-dependent regulation of transcription" ($p < 10^{-5}$). Coincident with this, three families of TFs are enriched ($p < 0.001$) in the activated CSR: AP2/EREBP, NAC, and WRKY (Table S2).

CSRs Show Cell Type Specificity

We hypothesized that the whole-root CSRs would be present in many, if not all, cell types in the root. Using fluorescently activated cell sorting of plants expressing cell type-specific GFP reporters (Dinneny et al., 2008) coupled with microarray analysis, we profiled five different cell types (Col, Epi, Cor, End, and Stl) in response to low pH and −S. Plants were grown on standard media and then transferred either to media with a pH of 4.6 (normal is pH 5.7) or sulfur-deficient (−S) media. Expression of GFP reporters was not altered under stress conditions (Figure S1). We combined these data with that from two additional stresses, high NaCl and Fe deficiency (−Fe) (Dinneny et al., 2008), which covered the same cell types. We defined a stress-regulated gene as one with significantly altered expression ($|FC| > 1.5$, FDR $< 1 \times 10^{-4}$) under stress conditions compared to standard conditions. Surprisingly, when we examined the distribution of the CSR genes across cell types in −Fe, high NaCl, low pH, and −S, we found that the majority of CSRs show cell type specificity (Figure 1B). Moreover, these cell type-specific CSR genes do not all respond in the same cell type. For example, some respond in the Col and others in the Cor or End, depending on the stress (Figure 1C).

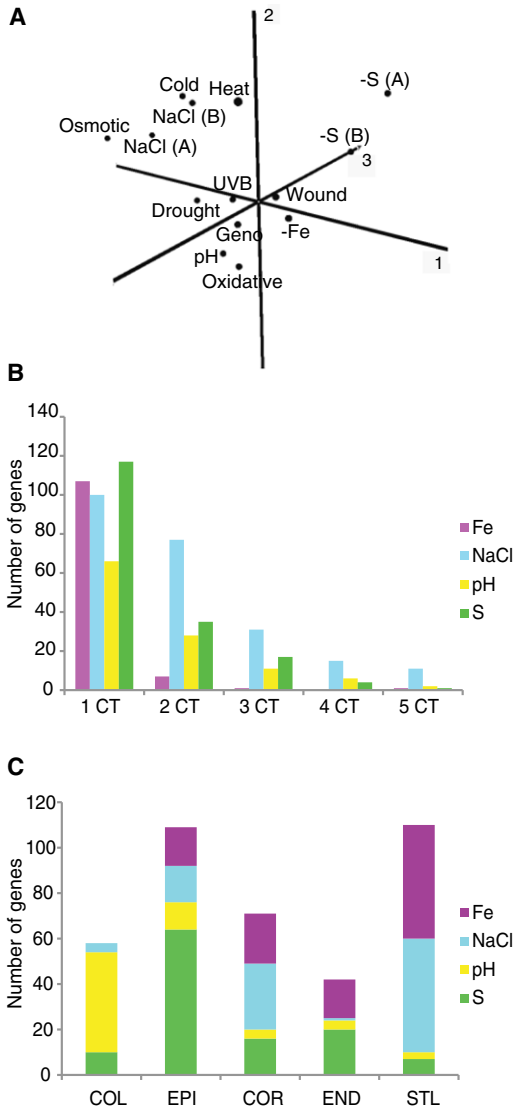


Figure 1. Stress Responses are Stress and Cell Type Specific

(A) PCA of GO categories significant in each of 14 stresses. Components 1, 2, and 3 explain 25.1%, 17.7%, and 11.7% of the variation, respectively. A, AtGenExpress; B, Benfey.

(B) The majority of whole-root CSRs respond in just one cell type, and this cell type differs for different stresses (C).

See also Figure S1.

ABA Responses Are Cell Type, Developmental Stage, and Stress Dependent

To further examine the cell type specificity of whole-root stress responses, we focused on those mediated by the plant hormone

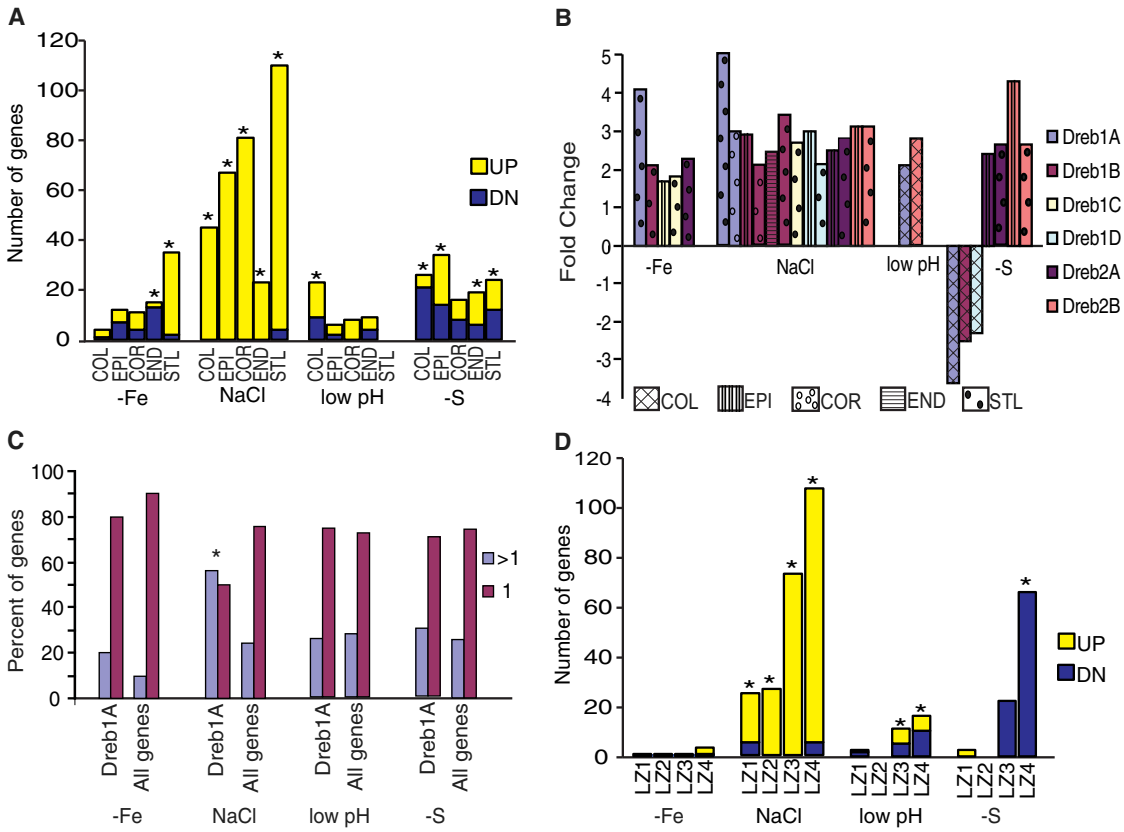


Figure 2. ABA-Mediated Stress Responses Are Stress and Cell Type Dependent

(A) ABA responses to salt are enriched in all cell layers, but enrichment is restricted to specific cell types under −Fe, low pH, and −S. Asterisk (*) indicates marker gene enrichment ($p < 0.005$).

(B) Response of the six DREB TFs to different stresses. Multiple bars indicate that the TF responds in more than one cell type; if not shown the TF is not differentially expressed in the stress.

(C) DREB1A putative targets are enriched (indicated by *; $p = 5.3 \times 10^{-5}$) in genes that respond to salt stress in more than one cell type but are regulated primarily in a cell type-specific manner under −Fe, low pH, and −S. >1, more than one cell type; 1, cell type specific.

(D) ABA marker genes are developmental stage and stress specific. Asterisk (*) indicates significance at $p < 0.001$. LZ1, apical meristem; LZ2, basal meristem; LZ3, elongation zone; LZ4, maturation zone.

See also Figure S2.

ABA because it is a well-documented CSR (Cutler et al., 2010), and ABA responses were enriched in the CSR genes ($p = 3.54 \times 10^{-11}$ in the activated CSR gene set; 22 of the 274 activated CSR genes were ABA marker genes; Table S2). First, we examined the cell type expression of ABA marker genes (Nemhauser et al., 2006) that significantly responded in at least 1 of the 14 conditions in our whole-root meta-analysis (Table S3; see Table S2 and Figure S2 for only the ABA marker genes that are also CSR genes). We find that ABA regulates responses to stress differentially across stresses and cell types in the root. ABA marker genes are primarily activated and enriched throughout all cell layers in the root under high NaCl, whereas ABA responses to −Fe, low pH, and −S are both activated and repressed, and enrichment is restricted to specific cell layers (Figures 2A and S2). Furthermore, the ABA response genes activated and repressed in each cell type and stress differ (Tables S2 and S3, and Figure S2).

Next, we examined the expression of master regulators of the ABA-signaling pathway within the cell type data (see Table S4 for all expression values in the cell type data set). The AP2 TFs DREB1A, 1B, 1D, 2A, and 2B control different components of the ABA pathway (Tuteja, 2007). Their expression within the five cell types assayed showed that although DREB1A, B, D, and DREB2A and B respond to NaCl in more than one cell type, they are regulated in a cell type-specific manner in at least one of the other stresses (Figure 2B). Consistent with this, putative targets of DREB1A are significantly enriched among genes that respond to NaCl stress in more than one cell type ($p = 5.3 \times 10^{-5}$) but are cell type specific under each of the other stresses (Figure 2C).

In addition to the cell type specificity, ABA marker genes show developmental stage specificity. We transcriptionally profiled four different developmental stages in the root after transfer to low pH or −S and combined these with similar data sets for high salt and −Fe. Similar to the results at cell type resolution, we find that ABA marker genes are differentially expressed both in developmental stages and stresses (Figure 2D; see Table S5 for developmental stage data set expression values).

Together, these results show that although ABA mediates responses to multiple abiotic stresses, these responses exhibit context-dependent cell type specificity.

Mutations in Cell Identity Regulators Lead to Altered ABA Responses

The cell type specificity of CSRs was surprising, and raised the question as to how this is controlled. We hypothesized that this specificity may be due to stress responsiveness of cell identity regulators, which we defined as genes with demonstrated roles in the determination or maintenance of a cell type. As a first test, we examined the expression under stress of a set of cell identity regulators at cell type resolution. We find that many cell identity regulators are differentially expressed under stress conditions. This occurs both in cell types known to be regulated by them and, occasionally, in cell types in which they have no documented role (Figure 3A and Table S6). For example *CAPRICE* (*CPC*), which is necessary for epidermal patterning, is induced by high NaCl in both the Epi and Stl (Figure 3A).

If cell identity regulators play a role in stress responses such as those mediated by ABA, we hypothesized that mutations in these factors could result in hypersensitivity or resistance to ABA. In addition to its role in stress responses, ABA functions in germination and early seedling development. We examined the germination and cotyledon emergence of six different cell identity regulator mutants, five of which had altered expression patterns under stress (Figure 3A). As shown in Figures 3B–3F, mutations in several cell identity regulators result in altered responses to exogenous ABA, either in germination, cotyledon emergence, or both. This is most likely not due to the developmental defects present in some of these mutants because *cpctry*, which has no root hairs, is not hypersensitive in either assay.

ABA Response Genes Are Direct and Indirect Targets of Cell Identity Regulators

Many of the cell identity regulators tested above encode TFs. We postulated that if these proteins play a role in ABA responses, their direct targets would include known ABA response genes. We tested this using SCR because the *scr* mutant is hypersensitive to ABA (Figures 3B–3D and 3F). To determine which genes regulated by SCR are direct targets, we performed chromatin immunoprecipitation (ChIP) of SCR followed by hybridization to an oligonucleotide microarray (ChIP-chip). We identified 181 putative SCR direct target genes (Table S7), several of which are ABA response genes (Figure 3G and Table S7). Indeed, GO category analysis showed that "Response to ABA stimulus" was enriched ($p < 0.001$) among the putative SCR direct targets (Table S7).

Because *wermyb23* and *gl2* mutants were also hypersensitive to ABA, we tested whether altered epidermal root hair patterning leads to differential ABA responses under stress. Using publicly available microarray data (Dinneny et al., 2008) from the super-hairy *wermyb23* and hairless *cpctry* double mutants, we identified stress-regulated hair patterning-dependent ABA marker genes (Figure 3H and Table S7). In line with our phenotypic results above, ABA marker genes were enriched ($p = 5.9 \times 10^{-12}$) among the genes misregulated under stress in the *wermyb23* mutant, but not in the *cpctry* mutant, which is not ABA hypersensitive. Taken together, our results show that cell identity regulators play a role in the root's response to stress, and suggest that these regulators are entry points for stress and developmental pathway interactions.

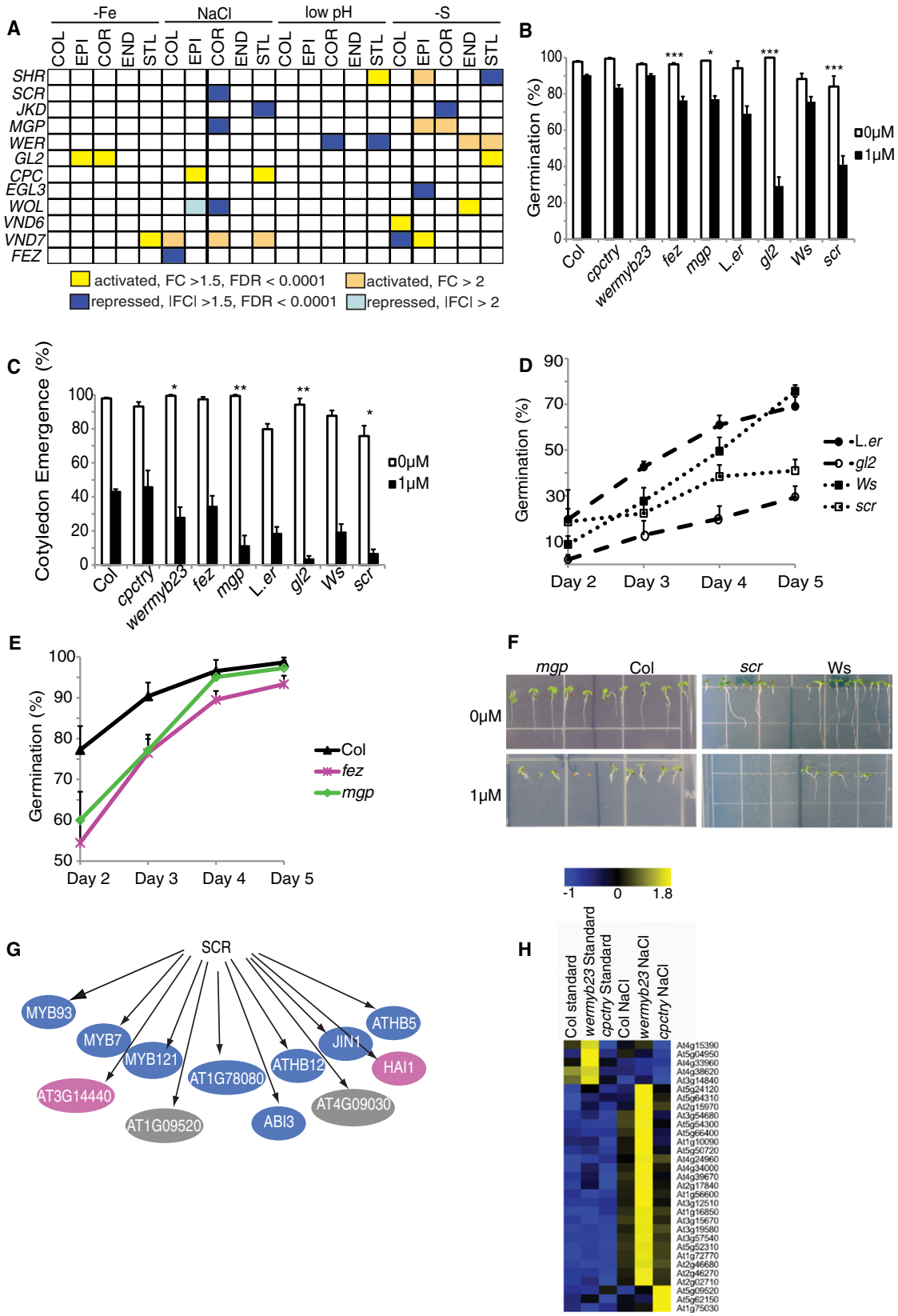
Response Centers Have Developmental Defects

The high degree of cell type specificity within the CSR genes from whole roots suggested that most genes responsive to stress in the five cell types profiled (the cell type gene set; see Table S4 for expression values) would also be cell type specific. Indeed, stress regulation depends both on the cell types and stress examined. The majority of stress-regulated genes in the cell type gene set respond in just one cell type, regardless of stress (Figure S3). Furthermore, the majority of responsive genes in this gene set are stress specific (Figure S3). However, the same gene can respond to different stresses in different cell types (Figure S3). We searched for a universal stress response at cell type resolution using the same meta-analytic methods as for whole roots. In line with the high degree of cell type and stress specificity, we found no evidence for such a response.

The most responsive cell type differs for each stress examined in the cell type gene set (Figure 4A). These "response centers" often correlate with a phenotypic change. For example the Col is the most responsive cell type under low pH, and the gravity-sensing response is altered under this stress (Figure 4B). Starch-filled plastids in the Col play a role in the gravity response in plants (Chen et al., 2002), and starch catabolism, metabolism, and biosynthesis are enriched GO categories in the Col under low pH (Figure 4C). Consistent with this, starch is absent from the Col 24 hr after transfer to low pH (Figure 4D).

Common Cell Type Stress Responses Identify Transcription Modules

Although we did not identify a universal stress response at cell type resolution, two biological responses, root hair morphogenesis and secondary cell wall biosynthesis, were enriched in at least three of the four stresses. Root hair development is altered under low pH, −Fe, and NaCl (Figure 5A) (Dinneny et al., 2008), and root hair marker genes are enriched in the Epi under all four stresses (Figure 5B). We hypothesized that looking for coexpression in response to stresses might identify genes with roles in specific developmental processes, particularly because regulators for each process are stress regulated (Figure 3A). Using k-means clustering, we grouped the cell type stress-regulated genes into 35 clusters (Table S8; see Supplemental Experimental Procedures for details). One cluster showed strong enrichment ($p = 9.66 \times 10^{-40}$) for root hair marker genes, including *GLABRA2* (*GL2*), a regulator of epidermal cell identity (Tominaga-Wada et al., 2009). We tested genes in this cluster to determine if they were regulated by *GL2* using quantitative RT-PCR (qRT-PCR) in the *gl2* mutant (Table S9). Of the 12 genes tested, 8 were differentially expressed in the mutant (Figure 5C). Many of these, including *RHS11* and *EXP7*, had not previously been shown to be regulated by *GL2*. We used a similar strategy with the bHLH TF *At5G58010*, which regulates root hair development both in *Arabidopsis* and *Lotus japonica* (Karas et al., 2009) and is also expressed in this cluster. Of the 12 genes tested by qRT-PCR in a T-DNA insertion line of *At5G58010*, 7 were misregulated (Figure 5C). These included known root hair regulators


A
-Fe
NaCl
low pH
-S
COL
EPI
COR
END
STL
SHR
SCR
JKD
MGP
WER
GL2
CPC
EGL3
WOL
VND6
VND7
FEZ
activated, FC >1.5, FDR < 0.0001
activated, FC > 2
repressed, |FC| >1.5, FDR < 0.0001
repressed, |FC| > 2
B
Germination (%)
0μM
1μM
Col
cpctry
wermyb23
fez
mgp
L.er
gl2
Ws
scr
C
Cotyledon Emergence (%)
D
Germination (%)
Day 2
Day 3
Day 4
Day 5
L.er
gl2
Ws
scr
E
Germination (%)
Col
fez
mgp
F
mgp
Col
scr
Ws
0μM
1μM
G
SCR
MYB93
MYB7
MYB121
AT3G14440
AT1G09520
AT1G78080
ABI3
ATHB12
AT4G09030
JIN1
ATHB5
HAI1
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1.8
Col standard
wermyb23 Standard
cpctry Standard
Col NaCl
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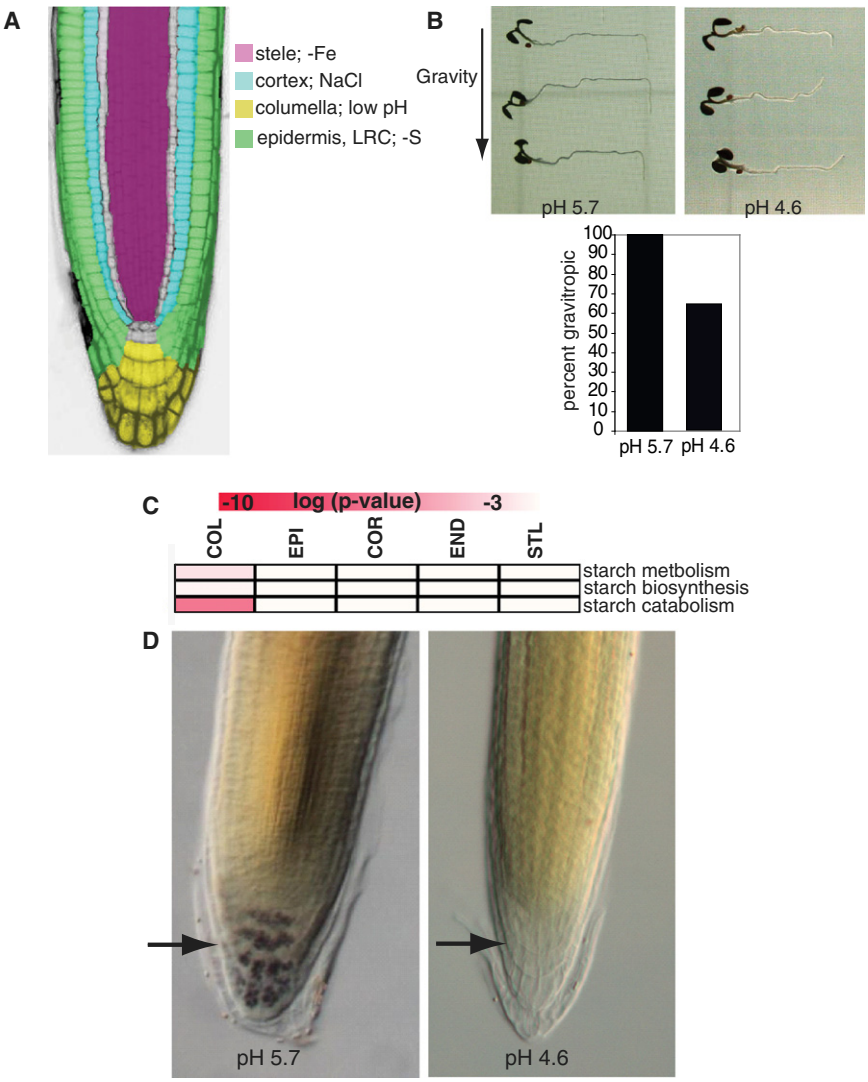

Figure 4. The Low pH Stress Response Center Correlates with Developmental Changes

(A) The most responsive cell type differs for each of the four stresses examined.

(B) Defective gravitropic response 24 hr after transfer to low pH.

(C) Heat map showing that the GO categories starch catabolism, metabolism, and biosynthesis are enriched in the Col under low pH. Red indicates level of enrichment.

(D) Most starch granules are absent from the Col 24 hr after transfer to low pH. Arrows point to the Col.

See also Figure S3.

such as *COBL9* and *RHS11* as well as genes with no known role in root hair development, such as the MYB TF *AT5G06800*.

Genes necessary for secondary cell wall biosynthesis (Persson et al., 2005; Brown et al., 2005) are enriched in the Stl in −Fe, NaCl, and −S (Figure 5D). We identified one cluster (cluster 5, Table S8) with strong enrichment ($p = 3.39 \times 10^{-33}$) of these genes. Cluster 5 contains a transcription module involving the protoxylem cell identity regulator *VND7* (Kubo et al., 2005), which is regulated at the cell type level in −Fe, high NaCl, low pH, and −S. qRT-PCR showed that multiple genes in this cluster were significantly up- or downregulated in a *35S::VND7* line (Figure 5D). Several of these have not previously been shown to be regulated by *VND7* and may be components of the secondary cell wall transcription network. Coexpression analyses of common cell type stress responses may be a good predictor for identifying genes downstream of cell identity regulators in the developmental pathways they control.

Core Markers Suggest Cell-Cell Communication in Stress Responses

Because cell-cell communication is vital for proper patterning in roots, we examined genes enriched in one cell type relative to all others under the same stress (Figures 6A and Figure S4; see Experimental Procedures). We identified 199 genes enriched in a cell type under all four stresses and the MS standard condition (Figure 6B and Table S10). Because these genes are enriched in a cell type regardless of environment, we define these as core markers (Dinneny et al., 2008). For example nine genes are enriched in the Stl compared to all other cell types in all environments tested (Figure 6B).

Because core markers are enriched in a cell type regardless of environment, we hypothesized that these genes may be important for cell identity. Indeed, genes known to regulate cell identity

Figure 3. Cell Identity Regulators Interact with the ABA-Signaling Pathway

(A) Cell identity regulators can be stress regulated both in cell types they regulate and in those they are not known to influence.

(B–F) Mutants in cell identity regulators result in altered responses to ABA. Error bars show SE. (B) Percent germination of seeds 3 (Col, *cpctry*, *wermyb23*, *fez*, *mgp*) or 5 (L.*er*, *gl2*, Ws, *scr*) days after transfer to light. L.*er* is the control for *gl2*; Ws for *scr*; Col for remaining mutants. (C) Percent fully expanded, green cotyledons 5 (Col, *cpctry*, *wermby23*, *fez*, *mgp*) or 6 (L.*er*, *gl2*, Ws, *scr*) days after transfer to light. For (B) and (C), stars denote significant differences between mutant and wild-type on 1 μM ABA: *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$. (D and E) Germination TC of hypersensitive mutants on 1 μM ABA 2–5 days after transfer to light. (D) Ws, *scr*, L.*er*, *gl2*; (E) Col, *fez*, *mgp*. (F) *mgp* and *scr* mutants are hypersensitive to ABA.

(G) SCR ChIP-chip reveals that ABA response genes (from Nemhauser et al. [2006] and GOs) are SCR direct targets. Blue indicates TFs, pink is enzymes, and gray is other.

(H) Expression of ABA marker genes under high NaCl in epidermal-patterning mutants *wermyb23* and *cpctry* shows that ABA marker gene expression is altered in the ABA hypersensitive *wermyb23* mutant.

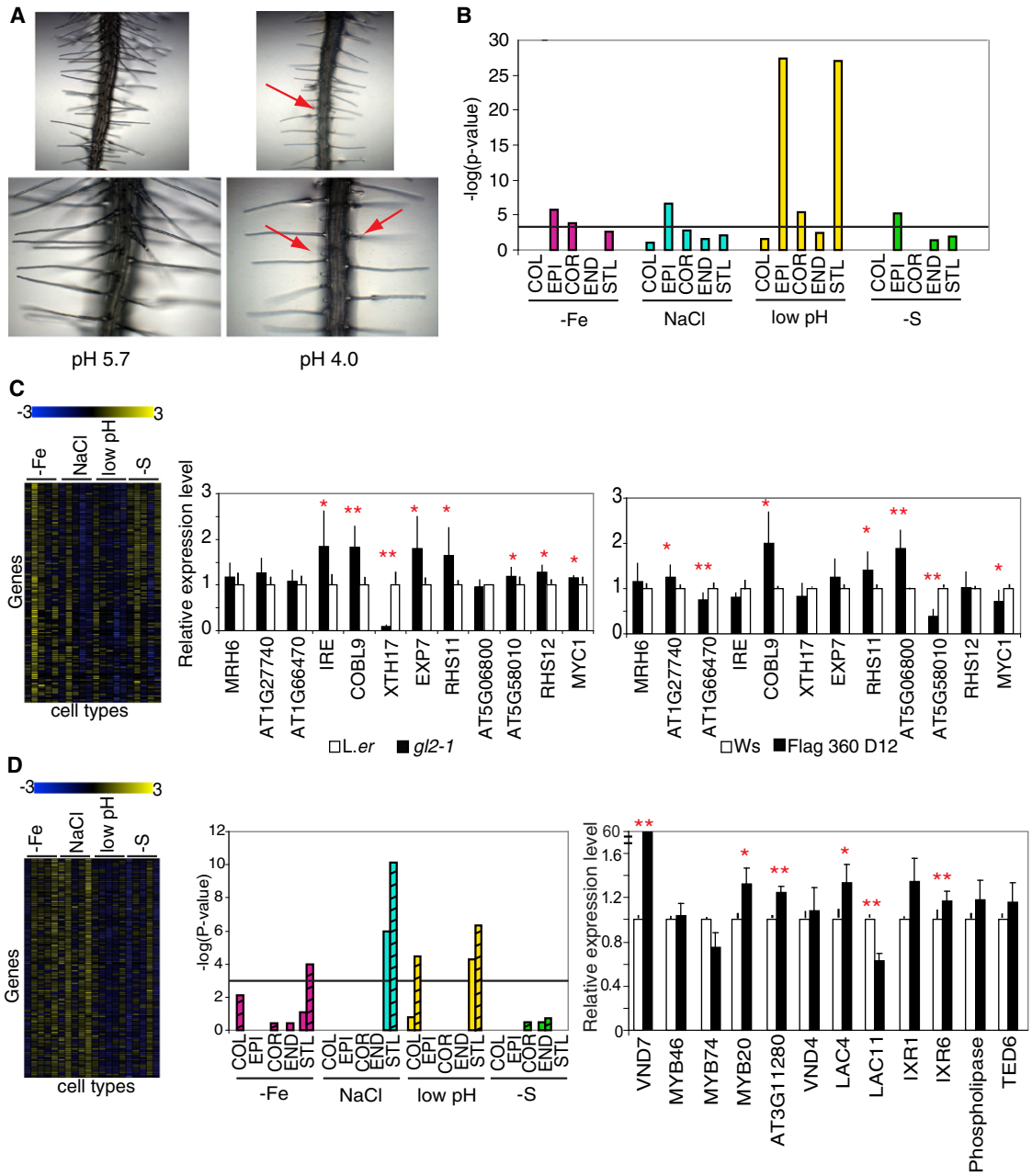

Figure 5. Cell Type Stress Data Reveal Components of Development-Associated Transcription Networks

(A) Root hair development is altered under low pH. Red arrows point to short, swollen root hairs.

(B) Root hair marker genes are enriched in the Epi under each stress. Black line marks $p < 0.001$.

(C) Root hair cluster with enrichment ($p = 9.66 \times 10^{-40}$) of root hair marker genes. Left view is of heat map showing fold change of expression for genes in the cluster for all cell types under each stress. Cell types are arranged as COL, EPI, COR, END, STL for each stress. Middle and right views show qRT-PCR of selected genes in the root hair cluster in the *gl2* mutant (middle) or *AT5G58010* T-DNA insertion line (right).

(D) Secondary cell wall biosynthesis cluster. Left view shows heat map with fold change of genes under each stress (cell type listed as in C). Middle view illustrates that secondary cell wall biosynthesis genes are enriched in the STL in three stresses. Striped bars indicate genes identified from Persson et al., 2005, and nonstriped, from Brown et al. (2005). Right view shows qRT-PCR of selected genes in this cluster in *35S::VND7*. Black indicates *35S::VND7*; white indicates Col. *$p < 0.05$ and **$p < 0.01$, one-tailed t test. Error bars denote SE.

are found among these markers. For example *LONESOME HIGHWAY*, *INCURVATA4*, and *ZWILLE* (*ZLL*) are all cell type markers in the Stl, and all play a role in vasculature development (Ohashi-Ito and Bergmann, 2007; Ochando et al., 2006; Tucker et al., 2008). Mutations in the core Col marker *PLETHORA1* (*PLT1*) result in extra Col cells (Aida et al., 2004), whereas overexpression of the Col marker *IAA20* causes the Col not to differentiate and leads to agravitropism (Sato and Yamamoto, 2008).

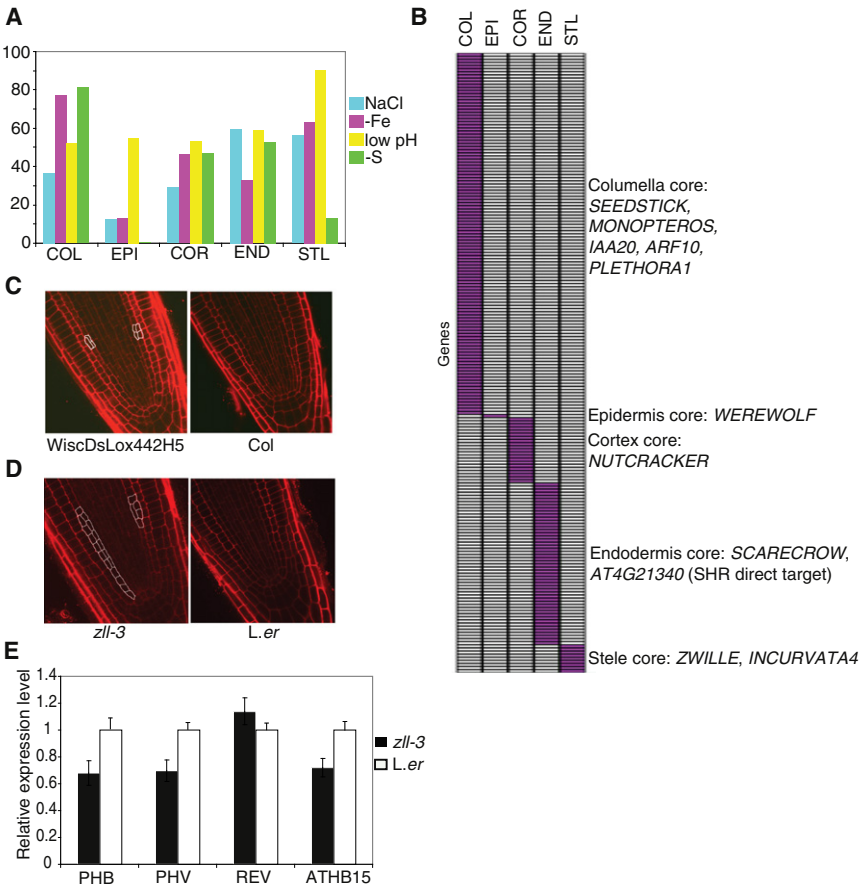

Figure 6. Stress-Enriched Genes Reveal Core Markers and Cell Identity Regulators

(A) The Epi is the most plastic cell type because it shows the least conservation between standard and stress conditions. Percent conservation of enriched genes between standard and stress conditions for each cell type is shown.

(B) Many cell identity regulators are found within the core markers.

(C and D) Mutations in the core markers *MYB36* (C) and *ZLL* (D) identify regulators of radial patterning.

(E) qRT-PCR showing that HD-ZIP genes *PHB*, *PHV*, and *ATHB15* are repressed in roots of the *zll–3* mutant. Error bars show standard deviation. See also Figure S4.

The TFs SHORTROOT (SHR) and SCARECROW (SCR) are necessary for the proper patterning of the End and Cor, and two SHR direct targets, an uncharacterized bHLH (*At4g21340*) (Sozzani et al., 2010) and the TF *NUTCRACKER* (Sozzani et al., 2010; Levesque et al., 2006; Cui et al., 2007), are cell type markers in the End and Cor, respectively. Furthermore, eight SHR and SCR direct or indirect targets were identified in the endodermal core marker genes (Sozzani et al., 2010).

Surprisingly, although core markers are enriched in a cell type under all conditions, they respond to stress in many different cell types (illustrated for selected markers in Figure 7A, and in Table S10 for all markers). However, their expression level is always higher in the core cell type compared to the cell type in which they respond to stress (Figure 7B and Table S10). For example, expression of *At4g05170*, a bHLH TF and core endodermal marker, is significantly activated in the Cor under −Fe compared to standard MS (FC = 3.8, FDR < 0.0001). However, its absolute expression value in the Cor under −Fe is 2.9, compared to 14.9 in the End under −Fe (Table S10). Thus, although core markers may respond to stress in different cell types, their expression levels are higher in the core cell type, suggesting some mechanism by which relative expression levels are communicated.

Core Markers Have Functional Relevance

Because many of the core marker genes are necessary for root patterning, we reasoned that this data set may contain previously undescribed root-patterning genes. Indeed, we found that mutations in two core markers, *MYB36* and *ZLL/PINHEAD/ARGONAUTE10 (AGO10)*, led to deviations from the typical wild-type root organization (Figures 6C and 6D). None of the stresses examined resulted in altered root radial patterning; thus, mutants were examined under standard conditions. A T-DNA insertion line (WiscDsLox442H5) of the core endodermal marker *MYB36* resulted in increased divisions in the ground tissue, although with low penetrance (6 of 26 plants; Figure 6C). Although *AGO10* is a core marker for the Stl, we found extra divisions in the ground tissue of the *zll–3/ago10* mutant compared to wild-type in approximately 54% (14 of 26) of plants examined (Figure 6D). Mutations in *AGO10* result in elevated levels of miR165/166 in stems and leaves and a consequent reduction in their target genes, the HDIII-ZIP TFs *PHABULOSA* (*PHB*), *PHAVOLUTA* (*PHV*), and *REVOLUTA (REV)*, and *ATHB-15* (Liu et al., 2008). We tested whether these target genes were downregulated in roots of *zll–3*. Consistent with their expression in stems and leaves, expression levels of *PHB*, *PHV*, and *ATHB-15* are downregulated in *zll–3* roots (Figure 6E). Together, these results suggest that core markers are important for root function and development.

DISCUSSION

Stress responses in multicellular organisms require the coordination of thousands of genes and regulatory networks. This transcriptional reprogramming differs by cell type and is required for an organ's developmental response to changing environmental conditions. In this report we transcriptionally profiled the response to two stresses in five cell types, four developmental stages, and different time points in the *Arabidopsis* root. We combined these data with two additional high-resolution data sets and 14 conditions in the whole root to form a comprehensive data set of the effects of abiotic stress on the root. Our results reveal a layer of complex gene regulation within cell types under

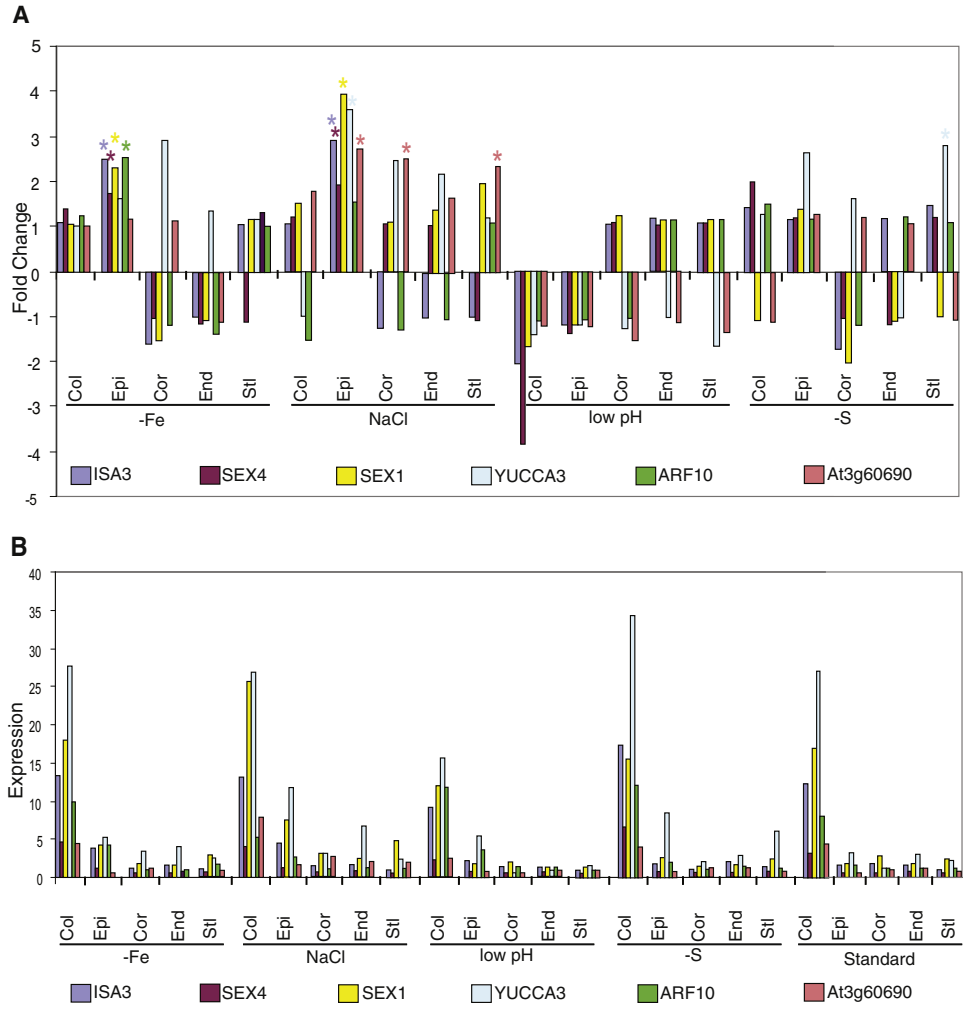

Figure 7. Core Markers Are Stress Responsive but Are Most Highly Expressed in the Core Cell Type

Fold change with respect to standard conditions (A) and expression values (B) for all cell types and stresses of six core marker genes in the Col. Several genes significantly (FC > 1.5, FDR < 0.0001) respond to a stress in cell types other than the Col (indicated by a star) (A), but their expression values are highest in the Col compared to all other cell types for a given condition (B). *At3g60690* is an auxin-responsive gene. See also Figure S4.

environmental stress and suggest that cell identity regulators play an important role in stress responses.

A Universal Stress Response in the Whole Root?

The idea of a universal stress response in plants is compelling because the identification of such a response could lead to the development of crops able to withstand many types of harsh environmental conditions. Understanding whether the root has a universal stress response depends on the resolution (whole root or cell type) and whether genes or functions are examined. Having explored 14 conditions in the whole root and 4 at cell type resolution, we found little evidence for a universal stress response in either data set. However, we identified a set of CSR genes that respond to multiple stimuli, in agreement with other studies (Ma and Bohnert, 2007; Walther et al., 2007; Swindell, 2006). Of the CSR genes, 46 were previously identified as members of a universal stress response cluster that examined 4 abiotic and multiple biotic stresses (Ma and Bohnert, 2007), whereas 15 of them were found among 26 root stress-general response genes (Swindell, 2006).

Although CSRs are present in the whole root, these responses cannot be generalized at cell type resolution because the majority are cell type specific. This is in agreement with the different functional responses of cell types to stress and suggests that the mechanisms underlying CSRs in plants may be fine-tuned at cell type resolution for each stress. This is analogous to animal systems, in which the expression level of cellular stress response genes and the pathways activated by TFs in this response can differ in different cell types (Kültz, 2005), suggesting a convergent evolutionarily theme of context-dependent stress specificity.

Cell Identity Regulators Play a Role in ABA Responses

Insight into how CSRs are regulated at cell type resolution can be gained from analyzing expression of cell identity regulators under stress. ABA responses are both cell type and stress

dependent, and we find that several stress-responsive cell identity regulators interact with the ABA response pathway. ABA is not only a stress hormone but also plays a key role in developmental pathways throughout the plant, including senescence, embryogenesis, and lateral root development (Cutler et al., 2010). Because environmental responses often result in morphological changes within the plant, stress and developmental pathways are inextricably linked, yet the interactions between them are poorly understood. However, evidence is building that cell identity regulators have a role in stress responses, and that in turn, stress-responsive TFs may regulate developmental patterning. Defects in the Col core marker ARF10 lead to altered root cap formation (Wang et al., 2005), and the *cre1* mutant, which has altered xylem organization (Inoue et al., 2001), is hypersensitive to ABA at germination (Tran et al., 2007). Furthermore, the phosphate deficiency-induced gene *PDR2* is necessary for maintenance of the ground tissue regulator SCR under phosphate-deficient conditions (Ticconi et al., 2009), whereas a putative direct target of the iron-responsive TF POPEYE is the SHR direct target MGP (Long et al., 2010). In addition the heat-shocked protein SCHIZORIA is required for stem cell maintenance (Pernas et al., 2010; ten Hove et al., 2010), directly implicating a stress-responsive TF in root patterning. We suggest that one role of cell identity regulators in the root is to interact with different stress response pathways.

Core Markers Reveal Root-Patterning Regulators

We identified a set of genes (core markers) that are always expressed at higher levels in the core cell type relative to all other cell types, regardless of environment. Many of these core markers are cell identity regulators. We have identified a role in radial patterning for the core Stl marker *ZLL*, a member of the AGO protein family. *ZLL* promotes leaf adaxial identity (Liu et al., 2008) and shoot apical meristem (SAM) maintenance during embryogenesis (Tucker et al., 2008). We find that *zll-3* mutants display increased divisions in the ground tissue in the root. Because *ZLL* is only expressed in the vasculature in the root, this suggests that *ZLL* regulates ground tissue patterning in a non-cell-autonomous manner. This is consistent with a previous report demonstrating that *ZLL* maintains SAM stem cells from the vascular primordium (Tucker et al., 2008). Levels of miR165/166 are elevated in leaves and the SAM of *zll* mutants. Levels of the HD-III Zip genes *PHB*, *PHV*, and *REV* are correspondingly reduced, suggesting that ZLL genetically represses miR165/166 (Liu et al., 2008). Recently, Carlsbecker et al. (2010) showed that the ground tissue-patterning regulators SHR and SCR activate miR165a and miR166b in the End. These microRNAs then move back into the Stl, and the resulting microRNA gradient represses HD-ZIP TFs in the End and Stl periphery, patterning the cell types of the xylem. *ZLL* is not affected in either a *SHR* or *SCR* induction TC (Sozzani et al., 2010) and, thus, appears to act in a SHR- and SCR-independent manner. Interestingly, mutations in *AGO1*, the closest homolog to *ZLL* in the *AGO* family, also lead to an increase in ground tissue layers in the root (Miyashima et al., 2009), though the mechanism behind this is not yet established. Recently, ZLL was shown to be a negative regulator of AGO1 at the protein level (Mallory et al., 2008), and the two genes are known to act redundantly in specific pathways. These data suggest that *ZLL* may regulate ground tissue patterning through a SHR and SCR-independent, non-cell-autonomous, posttranscriptional mechanism.

Cell-Cell Communication Is Important for the Stress Response

Cell-cell communication is a vital part of root development. Because the expression level of core markers is highest in the core cell type regardless of environment, this raises the possibility that cell-cell communication allows cell types in the root to determine the relative concentration of specific genes. Because many core markers are cell identity regulators, this may be another point of interaction between root developmental pathways and stress responses.

Together, our results highlight the complexity of gene regulation within cell types under stress and demonstrate the power of multiple genome-wide analyses from different environmental stimuli to uncover root-patterning factors and regulatory associations within cell types. Our data suggest that cell identity regulators play dual roles in stress and development. We speculate that this may contribute to the enormous phenotypic plasticity observed in the plant kingdom.

EXPERIMENTAL PROCEDURES

Plant Materials and Growth Conditions for Microarrays

Columbia-0 (Col-0) was used for all microarray experiments. Seeds were surface sterilized with 50% bleach and 0.1% Tween for 5 min and then rinsed three times with sterile water. Seeds were stratified at 4°C for 48 hr before sowing and plated on nylon mesh on agar for all experiments as described (Dinneny et al., 2008). High NaCl, −Fe, and MS standard media are as described (Dinneny et al., 2008). −S media have a similar concentration of nutrients (with the exception of S) as the MS standard (full description in Supplemental Experimental Procedures). Low pH media are 1X concentration MS salt mixture (Caisson laboratories), 3 mM DMG (Sigma), 1% sucrose, 1% agar, and adjusted to pH 4.6 with KOH. Separate standard media (pH standard) were used to compare to low pH for all experiments. The pH standard is equivalent to the low pH media with the exception that the pH was adjusted to 5.7.

ABA Assays

For radicle and cotyledon emergence assays, seeds of Col-0, Ws, L*er*, *scr4* (Ws control), *mgp* (Col-0 control), *fez-2* (Col-0 control), *wermyb23* (Col-0 control), *cpctry* (Col-0 control), and *gl2* (L*er* control) were cold treated for 48 hr, sterilized as above, and plated on 0 μM and 1 μM ABA plates. One micromolar ABA plates were 1X MS salt mixture, 0.05% MES, 1% agar, and 1 μM ABA (Sigma). Zero micromolar ABA plates were the same except an equivalent volume of ethanol was added in place of the ABA. Radicle emergence was scored using a Leica 6SE dissecting scope; cotyledons were scored as fully emerged if they were green and open past 90°. Experiments were repeated three times for *fez*, *wermyb23*, *cpctry*, and *scr*, and twice for *mgp* and *gl2* ($n \approx 60$/genotype/concentration/experiment, except *gl2* and *scr* in which $n \approx 30$). The average of experiments is shown as ±standard error (SE). Significance was tested using a one-tailed t test with equal variance.

FACS and GFP Reporter Lines

FACS and GFP reporter lines used for cell sorting are as described (Brady et al., 2007; Dinneny et al., 2008).

Sample Preparation for TC, Cell Type, and Longitudinal Data Sets

Sample preparation for the TC, cell type, and longitudinal data sets is as described (Dinneny et al., 2008) except that seedlings were transferred to either low pH and the pH standard or −S and fresh MS. A full description is in Supplemental Experimental Procedures. All low pH and −S microarray

data sets have been submitted to Gene Expression Omnibus (GEO) (SuperSeries GSE30166).

Sorting Effect Sample Preparation

Low pH samples for the effect of sorting were prepared as described (Dinneny et al., 2008). Cell sorting-affected probe sets are listed in Table S9.

Microarray and ChIP-chip Analysis

Meta-analysis

All computations for the meta-analysis were done using R. CEL files for AtGenExpress data sets were downloaded from TAIR. All arrays from each condition were background corrected and normalized together using RMA with the affy package. The RankProd package (Hong et al., 2006) with two classes was used for identifying differentially expressed genes within each TC. p values from RankProd were used to generate a combined p value from 14 treatments for each probe set. Combined p values were generated using Fisher's method (Fisher, 1932; Burguillo et al., 2010) in the survcomp package. FDR values were determined using the q-value package (Storey and Tibshirani, 2003) with the default settings. Because Fisher's method is asymmetric in the weight it gives to small numbers of significant values (Zaykin et al., 2002), significant probe sets had to meet two criteria: first, a combined FDR value <0.0001; and second, in at least three-fourths of the treatments, an FDR < 0.01. Thus, to be considered significant among 14 conditions in the whole root, a probe set with a combined FDR <0.0001 also had to have an individual stress FDR <0.01 in at least 11 of 14 stresses. For the meta-analysis at cell type resolution, a probe set had to have a combined FDR < 0.0001 and an individual treatment FDR < 0.01 in at least 15 of 20 conditions (5 cell types, 4 stresses = 20 conditions). All probe sets on the array were used in the meta-analysis.

Normalization and Identification of Differentially Expressed Probe Sets for Stress-Regulated Genes

All arrays were normalized and differentially expressed probe sets identified using a mixed-model ANOVA Perl script as described (Levesque et al., 2006). Arrays for each stress were normalized separately. See Tables S4 and S5 for expression values for cell type and developmental stage data sets. The Pearson correlation product was calculated for all replicates. A cutoff value of 0.91 was used for the low pH data set and 0.88 for the −S data set.

A list of 20,385 singleton probe sets was generated by eliminating both the Affymetrix probe sets that were predicted to hybridize to more than one locus and loci that were predicted to have multiple probe sets that detected expression (Table S9). This was based on the lookup table from Affymetrix published on May 29, 2008. Only probe sets that hybridized to nuclear genes were used for analysis. Differentially expressed probe sets for all TC and stress-regulated lists were identified using an |1.5|-fold change cutoff and an FDR of 1×10^{-4}. Differentially expressed probe sets for the stress-enriched lists were identified using a 1.5-fold change cutoff and an FDR of 1×10^{-4}. The exception was the –S-enriched probe sets for the Stl and Epi, for which a 2-fold change cutoff value was used.

Stress-regulated probe sets for the cell type and developmental stage data sets were identified by comparing each cell type or developmental stage under stress to the same cell type or developmental stage under standard conditions. Stress-enriched probe sets were identified by comparing each cell type to all other cell types under the same condition (see Figure S4 for description of significant versus enriched probe sets). Heat maps were created using TMV microarray software (http://www.tm4.org). For all cell type analyses except k-means clustering, sorting-affected probe sets were removed from the analysis for high NaCl, −Fe, and low pH (listed in Table S9). In the text "responsive" always refers to those genes that meet the significance cutoff.

DREB1A Target Analysis

DREB1A target analysis was as described (Dinneny et al., 2008). A full description is in Supplemental Experimental Procedures.

GO Enrichment Analysis

GO analysis was preformed using the ChipEnrich program as described in Orlando et al. (2009). Statistical significance for each GO category was determined using the hyper-geometric distribution as described (Brady et al., 2007; Dinneny et al., 2008; Orlando et al., 2009).

Stress-Regulated Hair Patterning-Dependent ABA Marker Genes

From results in Dinneny et al. (2008), we obtained a list of genes that are differentially expressed (FDR < 0.0001, |FC| > 1.5) under NaCl and dependent on hair patterning. This list was then used to query the ABA marker genes from Nemhauser et al. (2006).

qRT-PCR

Procedures for qRT-PCR are as described (Dinneny et al., 2008; Tsukagoshi et al., 2010). A full description is in Supplemental Experimental Procedures.

ChIP-chip Procedure and Analysis

Procedures for ChIP-chip and analysis were as described (Sozzani et al., 2010; Long et al., 2010; Tsukagoshi et al., 2010; Busch et al., 2010). Two biological replicates each of homozygous pSCR:SCR:GFP *scr-4* and Columbia (Lehle) (control) were processed. A full description is in Supplemental Experimental Procedures. ChIP-chip data have been submitted to GEO as part of SuperSeries GSE30166.

ACCESSION NUMBERS

Coordinates have been deposited in the GEO with accession code GSE30166. All low pH and −S microarray data sets have been submitted to GEO (SuperSeries GSE30166). ChIP-chip data have been submitted to GEO as part of SuperSeries GSE30166.

SUPPLEMENTAL INFORMATION

Supplemental Information includes Supplemental Experimental Procedures, four figures, and ten tables and can be found with this article online at doi:10.1016/j.devcel.2011.09.009.

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